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13. ABSTRACT (Maximum 200 words)

The results of the current project to date show various approaches that can be used to improve the efficiency of bicarbonate-activated hydrogen peroxide for CWA and BWA destruction. Kinetic and spectroscopic results support the formation of peroxymonocarbonate ion (HCO_4^-) as the oxidant in the catalytic reactions. Variation of bicarbonate source and the cosolvent can allow optimization of substrate solubility and oxidation rates for applications in chemical warfare agent decontamination. Use of surfactants and microemulsions in place of non-aqueous cosolvents has proven to be effective for mustard simulants. Surfactant-catalyst formulations have been developed that enhance oxidation reaction rates up to 20-fold or more over usual cosolvent-based BAP formulations. Biochemically-relevant oxidations have been studied in view of the possible combination of enzymatic and chemical decontamination approaches as well as the potential of BAP for BWA applications. Recent work has determined the enhancement of peroxide biocidal activity in the BAP system via direct studies of bacterial culture survival rates under various treatments.

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Enclosure 1

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(Continuation Sheet)

Statement of problem studied:

Introduction

The oxidative destruction of chemical warfare agents (CWA) and biological warfare agents (BWA) is a promising approach for decontamination and demilitarization applications. Various oxidizing agents can be used to oxidize sulfides to sulfoxides and sulfones, and some of these have been investigated for simulants related to mustard, HD, and HD itself. We have been investigating the activation of peroxide by the bicarbonate ion as an effective approach to CWA oxidation and perhydrolysis. The H_2O_2/HCO_3^- oxidation method (or *BAP* for "bicarbonate-activated peroxide") has several advantages for potential application in CWA and BWA decontamination.

Summary of work during project period

The results of the current project show various approaches that can be used to improve the efficiency of bicarbonate-activated hydrogen peroxide for CWA and BWA destruction. Kinetic and spectroscopic results support the formation of peroxymonocarbonate ion (HCO_4^-) as the oxidant in the catalytic reactions. Variation of bicarbonate source and the cosolvent can allow optimization of substrate solubility and oxidation rates for applications in chemical warfare agent decontamination. Use of surfactants and microemulsions in place of non-aqueous cosolvents has proven to be effective for mustard simulants. Surfactant-catalyst formulations have been developed that enhance oxidation reaction rates up to 20-fold or more over usual cosolvent-based BAP formulations. Biochemically-relevant oxidations have been studied in view of the possible combination of enzymatic and chemical decontamination approaches as well as the potential of BAP for BWA applications. Recent work has determined the enhancement of peroxide biocidal activity in the BAP system via direct studies of bacterial culture survival rates under various treatments.

List of manuscripts:

(a) Published:

"Mechanism of Peroxymonocarbonate Oxidations of Sulfides," Deon Bennett, Huirong Yao, and David E. Richardson, *Inorg. Chem.***2001**, *40*, 2996.

"Equilibria, Kinetics, and Mechanism in the Bicarbonate Activation of Hydrogen Peroxide: Oxidation of Sulfides by Peroxymonocarbonate," David E. Richardson, Huirong Yao, Karen M. Frank and Deon A. Bennett, *J. Am. Chem. Soc.* **2000,** *122*, 1729.

"Epoxidation of Alkenes with Bicarbonate-Activated Hydrogen Peroxide," Huirong Yao and David E. Richardson, *J. Am. Chem. Soc.* **2000**, *122, 3220.*

(c) Scientific meeting presentations:

Regino, C. A. S.; Yao, H.; Johnson, J. V.; Nichols, L. S.; Méndes, T. J.; Richardson, D. E. "Catalytic Oxidation of Biomolecules by Peroxymonocarbonate: A Reactive Oxygen Species in Biochemistry?" *ACS National Meeting*, Washington, DC, August 20-25, **2000**.

Bennett, D. A.; Yao, H.; Frank, K. M.; Xu, C.; Richardson, D. E. "Kinetics and Mechanism of Bicarbonate-Catalyzed Oxidation of Arylsulfides by Hydrogen Peroxide" *ACS National Meeting*, Washington, DC, August 20-25, **2000**.

Yao, H.; Richardson, D. E. "Epoxidation of Alkenes by Bicarbonate-Activated Hydrogen Peroxide" *ACS National Meeting*, Washington, DC, August 20-25, **2000**.

Yao, H., Richardson, D. E. "Oxidations by Bicarbonate-Activated Hydrogen Peroxide: Equilibria, Kinetics and Micellar Effects", Inorganic Seminar, Department of Chemistry, University of Florida, FL, September, **2001**.

Bennett, D. A.; Yao, H.; Richardson, D. E. "Kinetics And Mechanism for the Epoxidation of Alkenes by Peroxycarbonate" National Meeting of American Chemical Society, Chicago, IL, August, **2001**.

Bennett, D. A.; Yao, H.; Richardson, D. E. "Kinetic Study for the Epoxidation of Alkenes by Peroxycarbonates" Florida Catalysis Conference, Palm Coast, FL, April, **2001**.

Regino, C.; Yao, H., Richardson, D. E. "Oxidation of Biomolecules by Peroxymonocarbonate, a New Reactive Oxygen Species Formed from Hydrogen Peroxide and Bicarbonate" Florida Catalysis Conference, Palm Coast, FL, April, **2001**.

(d) Submitted:

"Methionine Oxidation by Peroxymonocarbonate, A Reactive Oxygen Species Formed from Bicarbonate and Hydrogen Peroxide," David E. Richardson, Celeste Regino, and Huirong Yao, submitted *Free Radicals in Biology and Medicine* (in revision).

Scientific personnel:

Huirong Yao (postdoctoral), Celeste Regino (graduate student), Deon Bennett (graduate student), Andrew Burke (graduate student), Dan Denevan (graduate student), David E. Richardson (PI)

Report of inventions:

none

Scientific progress and accomplishments:

Mechanism of Peroxycarbonate Formation and Substrate Oxidations

The proposed mechanism for sulfide oxidation by BAP is given in eqs 1 - 4.

$$H_2O_2 + HCO_3^- = H_2O + HCO_4^- \quad K_{eq} \qquad (1)$$
$$HCO_4^- + RSR' \rightarrow HCO_3^- + RS(O)R' \quad k_1 \quad (2)$$
$$HCO_4^- + RSR' + H_2O_2 \rightarrow HCO_3^- + RS(O)R' + H_2O_2 \quad k_2 \qquad (3)$$
$$HCO_4^- + RS(O)R' \rightarrow HCO_3^- + RS(O)_2R' \quad k_{sulfone} \quad (4)$$

The mechanism of the reaction in eq 1 has been studied by using ^{13}C NMR. In recent experiments, we used a double pH-shock method to first convert HCO_3^- to CO_2 then raise the pH to allow water and peroxide to compete for the intermediate CO_2. The initial distribution of products HCO_3^- and HCO_4^- can be used to determine relative rate constants for hydration and perhydration, and the subsequent equilibration kinetics can then be modeled as a function of pH. A summary of our current best-fit model is shown below.

We have also studied the equilibration kinetics for eq 1 by ^{13}C-NMR in the presence of carbonic anhydrase or a zinc model compound for the active site of CA, 1,4,7,10-tetraazacyclododecanezinc(II) (Zhang & Van Eldik 1995). In both cases, the equilibration of eq 1 after mixing was complete in the time required to obtain the initial ^{13}C-NMR spectrum ($t1/2 < 60$ s vs. $t1/2 \tilde{2}$ 300 s in the absence of catalyst at 25 C), indicating significant catalysis of the formation of HCO_4^- when the dehydration reaction and, probably, the perhydration reaction are accelerated.

In order to obtain kinetic parameters for the catalytic effect of the zinc model complex on the formation of HCO_4^-

the equilibration kinetics for eq 1 were investigated in the presence of various concentrations of [([12]aneN4)Zn(II)][ClO4]2 at 10 C. The equilibrium constant Keq (eq 1) was calculated to be 0.46 ± 0.02 M^{-1} at 10 C from the relative peak intensities and the concentration of H_2O_2. The predicted first-order rate constant for the equilibration reaction in eq 1 is given by $k_{obs} = (k_f + k_{fcat}[ZnL])([H_2O_2] + Keq-1)$ in the presence of the zinc catalyst, where k_{fcat} is the third-order rate constant for the catalytic pathway. The observed pseudo-first order rate constant kobs has a linear relationship with the concentration of Zn(II) complex, as is shown in Figure 2. From the rate law and the concentration of H_2O_2, at 10 C the catalytic rate constant for the forward reaction k_{fcat} is 8.0×10^{-2} $M^{-2}s^{-1}$. The estimated t1/2 for equilibration of eq 1 at 25 C in the presence of 0.010 M Zn catalyst and 2 M H_2O_2 is ~40 s.

Figure 1

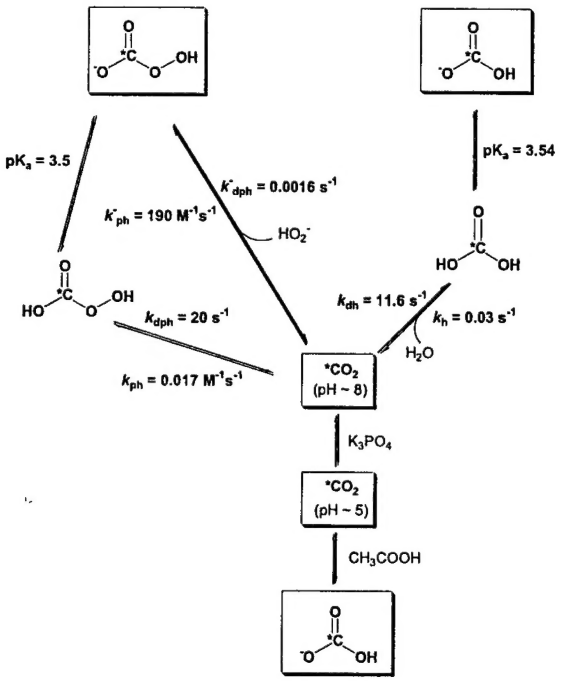
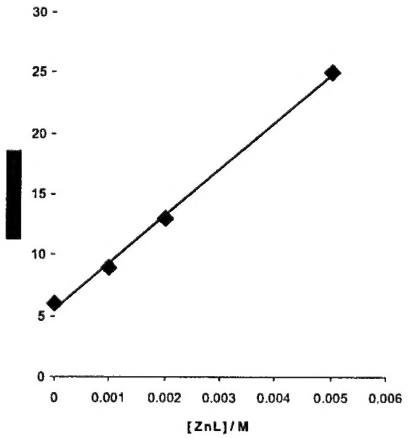


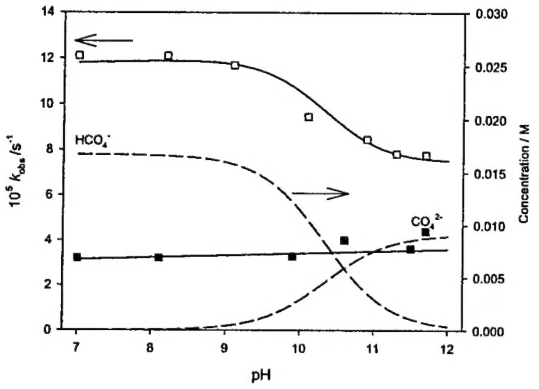
Figure 1



pKa of HCO_4^-

From the pH dependence of oxidation rates and ^{13}C NMR equilibrium experiments, the estimated pKa of peroxymonocarbonate was found to be ~ 10.6. As seen in Figure 3, although the reactivity decreases as HCO_4^- is deprotonated, CO_4^{2-} remains reactive with the simulant EPS (open squares in Figure 3). Thus, a broad range of pH values can be used to produce the optimal formulation for a universal decon system.

Figure 2



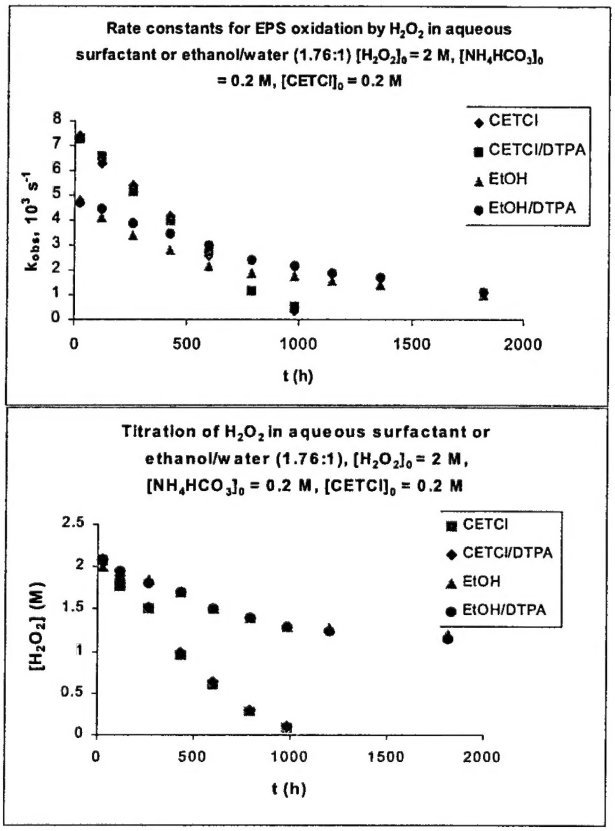
Lifetime of BAP Solutions

The instability of activated H_2O_2 solutions is a potential weakness for the development of stable peroxide-based decontamination systems usable after long term storage. Indeed, many strong oxidant solutions, such as hypochlorite, are subject to decomposition over time. In aqueous solution, H_2O_2 is thermodynamically unstable with respect to disproportionation into O_2 and H_2O. In addition, if H_2O_2 is activated through formation of a more reactive adduct the lifetime of active oxygen in the solution is usually diminished. We have completed a two month study to assess the storage stability of bicarbonate-activated peroxide vs. unactivated peroxide solutions.

Sample solutions of H_2O_2 were prepared in H_2O, CETCl/H_2O, and EtOH/H_2O, with added NH_4HCO_3 or $(NH_4)_2HPO_4$. In addition, EtOH/H_2O and CETCl/H_2O solutions with chelating agent DTPA were also prepared for stability studies to reduce possible interference by trace metal impurities. The solutions were titrated with the standard iodometric method periodically. For the solutions of H_2O_2/NH_4HCO_3, the rates for oxidation of ethylphenylsulfide were followed at the same time. The rate of ethylphenylsulfide oxidation and the concentration of H_2O_2 determined by iodometric titration are plotted as a function of time in Figure 4.

In general, H_2O_2 is less stable in solutions containing NH_4HCO_3 than the corresponding solutions buffered with $(NH_4)_2HPO_4$. This is especially obvious for CETCl surfactant solutions ($t_{1/2}$ of H_2O_2 = 20 days vs. > 360 days). The results suggest that the stability of H_2O_2 decreases by activation of H_2O_2 to form peroxymonocarbonate. The decay of the rates for ethylphenyl sulfide oxidation corresponds to the titration curves in EtOH/H_2O or CETCl/H_2O solutions with NH_4HCO_3. The decomposition of NH_4HCO_3 in EtOH/H_2O solution may account for the faster decay of oxidation rate comparing to the life time of H_2O_2 obtained from the titration curves. The decomposition of H_2O_2 slows down gradually in EtOH/H_2O with the loss of NH_4HCO_3 (presumably to NH_3, CO_2 and H_2O).
We conclude that H_2O_2 solutions with useful chemical reactivity are insufficiently stable to be stored for long periods without significant loss of oxidation capacity.

Figure 3



Biochemical oxidations by peroxymonocarboante

The kinetic parameters for the HCO_4^- oxidation of methionine were obtained previously ($k_1 = 1.0 \pm 0.3$ M^{-1} s^{-1}). At the concentration of bicarbonate in blood, it is estimated that peroxymonocarbonate formed in equilibrium with H_2O_2 will oxidize methionine up to two-fold more rapidly than plasma H_2O_2 itself. As an example of methionine oxidation in proteins, the bicarbonate-catalyzed H_2O_2 oxidation of α1-proteinase inhibitor has been investigated via its effect on elastase activity. The rate constant for HCO_4^- oxidation of α1-proteinase inhibitor (0.36 ± 0.06 M^{-1} s^{-1}) is comparable to that of met, suggesting that met oxidation is occurring.

We have studied the oxidation of lipids and model alkenes because of the possible role of membrane oxidation in biocidal activity. Oxidation of mono- and di-unsaturated fatty acids and model compounds results in epoxidation followed by hydrolysis. In addition, we investigated the oxidation of guanosine as a model for nucleic acids, RNA, and DNA. By HPLC-electrospray mass spectrometric analysis, BAP oxidation of guanosine produces a number of products, including the 8-oxo product used widely as a marker for DNA damage. In both general classes of compounds, H_2O_2 alone is essentially unreactive, but BAP is active for oxidations.

Development of Surfactant/Water Media for Oxidations and Acceleration of HCO_4^- Oxidations

We have done experiments designed to test the replacement of cosolvent in BAP oxidations with surfactants and microemulsions to achieve substrate solubility and increase reactivity. This work was based on the idea that

reducing the amount of organic components (e.g., cosolvents) in decon solutions would be desirable in some circumstances due to environmental and toxicity considerations. In addition, it would be possible to produce concentrated formulations using surfactants that would require only addition of water from local supplies, thereby reducing transportation and storage burdens.

Cationic surfactants proved to be excellent cocatalysts and solubilizing agents for oxidation of simulant sulfides (Figure 5) and alkenes. Figure 6 shows the dependence of k_{obs} for ethylphenylsulfide oxidation on concentration of HCO_3^- for three cationic surfactants, and Figure 5 shows that these solutions can be more active than ethanol/water with the same catalyst concentration.

Our results are consistent with the expectations from the ion-exchange model for micellar catalysis. Bromide ion competes effectively with HCO_4^- for the cationic interface, while chloride is more readily displaced. The linear dependence on HCO_3^- concentration is consistent with ion-exchange equilibria.

We have also produced reactive counterion catalysts that show exceptional activity in promotion of HCO_4^- oxidations ($k_1/k_0 = 4000$ vs ~200 to 300 in alcohol/water mixtures). One of these formulations is shown as "UF catalyst" in Figures 5 and 6. These are easily synthesized cationic surfactants with HCO_3^- counterions that react with bulk H_2O_2 to produce micelle-bound HCO_4^-, which has high reactivity with substrate at the micelle surface. In addition to the kinetic effect, cationic micelles have a thermodynamic preference for binding HCO_4^- over HCO_3^-, thereby increasing K_{eq} value for eq 1 and increasing available HCO_4^- for oxidations.

Figure 4

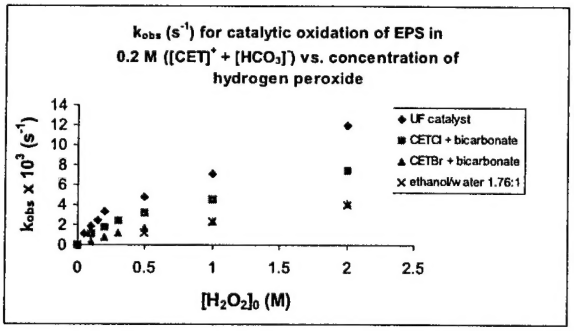
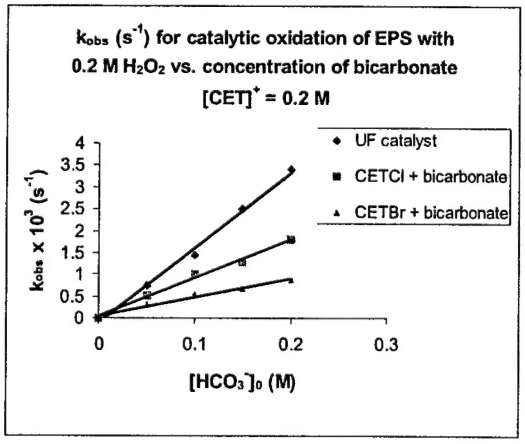


Figure 5



Biocidal Activity of Bicarbonate-activated Peroxide - Comparisons to Hypochlorite and Unactivated H2O2

We have suggested that the BAP system could be the foundation for a truly universal decontaminant encompassing both chemical and biological warfare agents. Therefore, we have studied its biocidal activity in collaboration with Dr. Hillman (U. Florida College of Dentistry). Our work on bacterial culture survival rates (including Salmonella and E. coli) has conclusively demonstrated that the BAP system has higher biocidal activity compared to the controls than H_2O_2 alone. For example, with $[HCO_3^-] = 0.10$ M, the biocidal activity of H_2O_2 is approximately two-fold higher that H_2O_2 with $[Cl^-] = 0.10$ M (Figure 7). We have taken care to insure reproducible conditions and results in these experiments.

In the course of our work, it became clear that the biocidal activity of hydrogen peroxide should be compared directly to that of hypochlorite, an effective, widely-employed biocide. We could not find direct, side-by-side comparisons of hypochlorite to hydrogen peroxide in the literature. The results for E. coli are shown in Figure 8. On a concentration basis, we find that the "killing rate constant" for H_2O_2 is approximately 50-fold less than that of hypochlorite (Figure 8). This difference in reactivity is significantly reduced through bicarbonate activation of H_2O_2, and this proposal considers peroxide-based formulations that could eliminate the difference. If all of the acceleration of killing with BAP is attributed to HCO_4^-, then the killing rate constant for HCO_4^- is comparable to that of OCl^-.

Figure 6

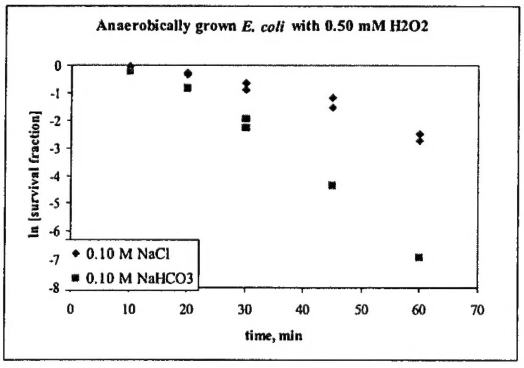
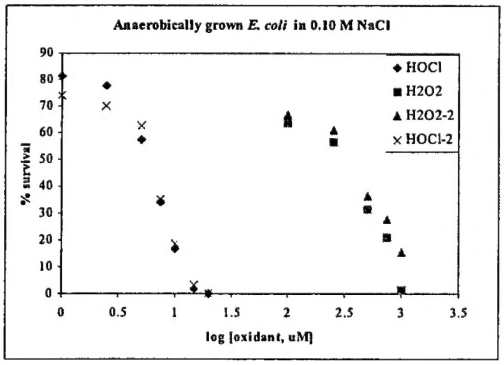


Figure 7



Technology transfer: none